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Preliminary

Lignin Peroxidase-Catalyzed Oxidation of a β-*O*-4 Model Compound of Lignin Carbohydrate Complex*[1]

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The ligninolytic systems of the white-rot fungi, including *Phanerochaete chrysosporium*, have been receiving much attention for its biotechnological application to pulp and paper industry[1,2]. Since the discovery of lignin peroxidase (LiP) as one of the potential lignin degrading enzymes[3,4], it has been shown to catalyze one-electron oxidations of a wide variety of compounds[5,6]. Quite recently, Hammel *et al.*[7] have provide the evidence that LiP is capable of depolymerizing lignin polymers without undergoing further repolymerization of the oxidized lignin.

However, the enzymatic oxidation of lignin carbohydrate complex (LCC) by the LiP system has little been investigated, although it is important to clarify the reaction mechanism for bond cleavages between lignin and carbohydrate moieties for biodelignification of lignocellulosics and kraft pulp. In this context, Tokimatsu *et al.*[8] have recently successfully synthesized four stereochemically pure diastereomeric LCC model compounds composed of β-*O*-4 lignin subsructure and methyl-β-D-glucoside.

For the enzymatic study of LiP-catalyzed oxidation of the LCC model compound (I, in Fig. 1), we cultivated *P. chrysosporium* (BKM-F-1769, ATCC 24725) according to the method of Kurosaka *et al.*[9] and the crude LiP enzyme preparation was obtained by concentration of the extracellular fluid. The reaction mixture contained 100 μg of the LCC model compound, 20 μl of 25 mM H_2O_2, 100 μl the LiP preparation, and 870 μl of 0.1 M sodium tartrate buffer (pH 3.0). The reaction was started by the addition of the enzyme solution and the mixture was incubated for 30 min at 37°C. The reaction products were extracted

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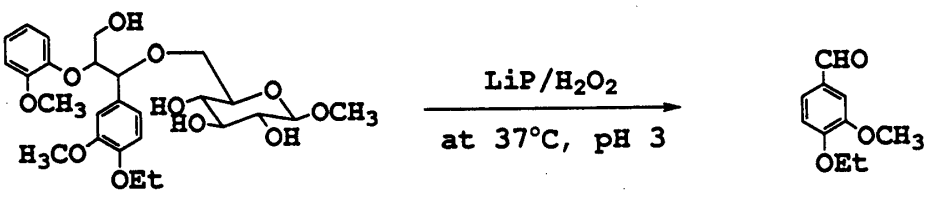

I II

Fig. 1. Catalyzed Degradation of the LCC Model Compound (I).
Et: —CH_2CH_3.

with ethyl acetate and the extracted and dried concentrate was submitted to GC-MS analysis after acetylation with acetic anhydride and pyridine.

The analytical result showed that 4-*O*-ethylvanillin (II) was detected from the complete reaction mixture and that no significant amounts of the product was detected from the control. The product was identified in comparison of its mass-spectral data and retention time on gas chromatogram with those of its authentic compound. This preliminary investgation reports on the first enzymatic oxidation of one of the *erythro* forms of the nonphenolic LCC model compounds with the LiP enzyme system, yielding the C_α-C_β bond cleavage product and the ether bond cleavage product as shown in Fig. 1. Since there are other unidentified products were observed by GC-MS analysis, further investigation is in progress in our laboratory.

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